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Growth of *Listeria monocytogenes* in egg salad and pasta salad formulated with mayonnaise of various pH and stored at refrigerated and abuse temperatures ☆

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Abstract

This study investigated and modeled the behavior of *Listeria monocytogenes* in egg salad and pasta salad as affected by mayonnaise pH (3.8, 4.2, 4.6, and 5.0) and storage temperature (4, 8, and 12 °C). At each storage temperature, *L. monocytogenes* was able to grow in both salads regardless of the mayonnaise pH. The lag-phase durations (LPD) of *L. monocytogenes* in egg salad ranged from 33 to 85, 15 to 50, and 0 to 19 h, and the growth rates (GR) ranged from 0.0187 to 0.0318, 0.0387 to 0.0512, and 0.0694 to 0.1003 $\log_{10}$ cfu/h at 4, 8, and 12 °C, respectively. The LPD of *L. monocytogenes* in pasta salad ranged from 210 to 430, 49 to 131, and 21 to 103 h, and GR ranged from 0.0118 to 0.0350, 0.0153 to 0.0418, and 0.0453 to 0.0718 $\log_{10}$ cfu/h at 4, 8, and 12 °C, respectively. The growth of *L. monocytogenes* was more rapid in egg salad than in pasta salad, indicating that a better growth environment for *L. monocytogenes* existed in egg salad. In both salads, the LPD decreased and the GR increased as the storage temperature increased. Mathematical models and response surface plots describing the LPD and GR of *L. monocytogenes* in both salads as affected by the mayonnaise pH and storage temperature were developed. The models confirmed that the growth of *L. monocytogenes* in egg salad and pasta salad was primarily promoted by higher storage temperatures and, secondarily, by higher mayonnaise pH. The conditions under which the models may be applied to estimate the growth of *L. monocytogenes* in both salads were identified.

Keywords: *Listeria monocytogenes*; Egg salad; Pasta salad; Mayonnaise; Model

1. Introduction

Listeria monocytogenes is a foodborne pathogen that is capable of growing at refrigerated temperatures. It causes an estimated 2500 cases of listeriosis and 500 deaths in the United States each year. Outbreaks of listeriosis have been linked to a variety of foods, especially processed meats, such as hot dogs, deli meats, and pâté, and dairy products made from unpasteurized milk (Bannister, 1987; Wang and Muriana, 1994; Centers for Disease Control and Prevention [CDC], 1998, 1999 and 2002). A multi-state outbreak of *L. monocytogenes* infections linked to eating turkey deli meat in the US in 2002 caused 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages. A total of 12.4 million kg of fresh and frozen ready-to-eat turkey and chicken products were recalled as a result of this outbreak (CDC, 2002).

Deli-type salads, such as meat, seafood, egg, and pasta salads, are ready for consumption without additional preparation or cooking. Deli salads, if not prepared and handled properly, are susceptible to contamination by *L. monocytogenes*. Gombas et al. (2003) reported that *L. monocytogenes* was positive in 2.36% (202/8549) and 4.70% (115/2446) of prepared deli salads (i.e., potato salad, tuna salad, pasta salad, and coleslaw) and seafood salads, respectively, with the majority of contamination

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levels ranging from 0.04 to 10 cfu/g. Data from the US and other countries showed that *L. monocytogenes* was positive in 9.9% deli salads that contained cooked seafood, meat, poultry, egg, cheese, or pasta as the primary salad ingredient (FDA/USDA/CDC, 2001). On the Belgian retail market, *L. monocytogenes* was isolated from 20.8% of ham salad, 26.9% of chicken salad, and 27.3% of fish and shrimp salad, with an overall incidence of 21.3% in mayonnaise-based salads (Uyttendaele et al., 1999). The possible contamination of *L. monocytogenes* in deli salads is one of the main causes of salad recalls in the US by the Food Safety and Inspection Service (FSIS) of USDA (FSIS-USDA, 2005; FDA, 2005). The USDA recommends that people at risk for listeriosis do not eat store-made salads such as ham salad, chicken salad, egg salad, tuna salad, or seafood salad (USDA, 2005b). The behavior of *L. monocytogenes* in mayonnaise-based seafood, ham, and potato salads stored at refrigerated and abuse temperatures have been examined (Hwang and Tamplin, 2005; Hwang, 2005). These studies showed that storage temperature was more significant than mayonnaise pH in influencing the behavior of *L. monocytogenes* in these salads. In addition, the behavior of *L. monocytogenes* in these salads was also affected by the food components in that a food component with a more favorable growth environment, e.g., high nutrient content and pH buffering capacity, was more readily to support the growth of this pathogen in salads stored at 4–12 °C (Hwang and Tamplin 2005; Hwang 2005). The objectives of this study were to examine and mathematically describe the behavior of *L. monocytogenes* in two salads made of different food components, egg and pasta, as affected by the pH of mayonnaise and storage temperature.

2. Materials and methods

2.1. L. monocytogenes strains and inoculum preparation

Eight strains of *L. monocytogenes* from the culture collection of the Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, USDA, were used in this study. These strains were originally obtained from the National Food Processors Association (Washington, District of Columbia, USA). These strains (serotype and source of isolation) were NFPA 7351 (1/2b, luncheon meat), NFPA 7389 (1/2b, luncheon meat), NFPA 7391 (1/2c, luncheon meat), NFPA 7427 (4d, luncheon meat), NFPA 7447 (1/2c, seafood salad), NFPA 7497 (4b, seafood salad), NFPA 7503 (1/2a, seafood salad), and NFPA 7601 (1/2b, seafood salad). A loopful of thawed frozen culture of each strain was transferred into 10 ml of brain–heart infusion (BHI, Difco Laboratories, Detroit, Michigan, USA) broth and incubated at 37 °C for 48 h to revive each strain. Each strain was further cultured by inoculating 0.1 ml of the cell suspension into 10 ml of a 2:1 mixture of BHI broth and a commercial Real mayonnaise, and incubated at 37 °C for 72 h to acclimate the cells to a low-pH environment (Leuschner and Boughtflower, 2001). The cell concentration of each strain was approximately 10^8 cfu/ml at the end of the incubation. One milliliter of cell suspension of each strain was combined and serially diluted in 0.1% peptone water to an inoculum level of approximately 10^5 cfu/ml.

2.2. Adjustment of mayonnaise pH

The initial pH of the mayonnaise used in this study was 3.8. The mayonnaise was adjusted to pH 4.2, 4.6, and 5.0 by mixing 200 g of mayonnaise with a 10% NaOH solution in 400 ml capacity stomacher bags (Spiral Biotech Inc., Norwood, Massachusetts, USA) using a BagMixer 400 Stomacher (Interscience, St. Nom, France). The pH-adjusted mayonnaise was stored at 4 °C for 24 h to allow for pH equilibration. The final pH of mayonnaise was confirmed by a Corning pH meter (model 430) fitted with a Corning '3-in-1' combo electrode (Corning Inc., New York, New York, USA).

2.3. Preparation, inoculation, and storage of egg salad and pasta salad

Large grade-A chicken eggs and dry Rotelle pasta (semolina flour enriched with niacin, ferrous sulfate, thiamin, mononitrate, riboflavin, and folic acid) were obtained from a local grocery store. Eggs were cooked in boiling water for 20 min, and placed in a refrigerator to cool. The eggs were peeled, and cut into small pieces in 237-ml capacity sterile polypropylene containers (Fisher Scientific, Pittsburgh, Pennsylvania, USA) using a sterile knife. Pasta was cooked in boiling water for 15 min according to the cooking instructions on the pasta box. The cooked pasta was drained, and cooled in a SterilGard hood (The Baker Company, Sanford, Maine, USA), and then kept in a refrigerator until use. To prepare the inoculated salad samples, 45 g of egg or pasta were placed into sterile polypropylene containers, and added with 5 ml of *L. monocytogenes* inoculum (10^5 cfu/ml). The inoculum and cooked egg or pasta were mixed thoroughly with a sterile spoon. Fifteen grams of mayonnaise that was previously adjusted to pH 3.8, 4.2, 4.6, or 5.0 was then mixed thoroughly with the inoculated egg or pasta. The initial levels of *L. monocytogenes* in egg or pasta salad were approximately 10^{2-3} cfu/g. Samples were prepared separately in triplicate. The containers were sealed with snap lids and stored at 4, 8, and 12 °C. The cell counts of *L. monocytogenes* in egg salad or pasta salad were enumerated during storage.

2.4. Enumeration of L. monocytogenes

At appropriate sampling intervals, samples from each treatment were enumerated for *L. monocytogenes* counts. Three grams of salad were placed into a 100-ml filter stomacher bag (Spiral Biotech Inc.), diluted 10-fold with

sterile 0.1% peptone water, and stomached for 1 min in a BagMixer 400 Stomacher. Additional dilutions, if needed, were prepared with sterile 0.1% peptone water. From appropriate dilutions, 0.05 ml of each dilution was spread-plated on duplicate modified Oxford agar (MOX, Oxoid Ltd., Hampshire, England). The plates were incubated at 35 °C for 48 h before black colonies surrounded by a black precipitation were counted as *L. monocytogenes*. If needed, the colonies were confirmed with API *Listeria* test strips (BioMerieux, Marcy l'Etoile, France).

2.5. Growth curve fitting and regression analysis

DMFit curve-fitting software (Baranyi, 2005) was used to fit *L. monocytogenes* counts ($\log_{10}$ cfu/g) versus storage time (h) to estimate the lag-phase durations (LPD, h) and growth rates (GR, $\log_{10}$ cfu/h) in egg salad and pasta salad. Means of LPD and GR of *L. monocytogenes* in salads formulated with various mayonnaise pH and stored at various temperatures were compared with a Tukey mean comparison test (SAS 9.1) at a significance level of 95%. The LPD or GR as functions of mayonnaise pH, storage temperature and their interaction were analysed by using the General Linear Model of the Statistical Analysis System (SAS) 9.1 for Windows (SAS Institute Inc., Cary, NC). The regression was fitted with the following quadratic function:

LPD (h) or GR ($\log_{10}$ cfu/h) $= \alpha + \beta 1$ (mayonnaise pH) $+ \beta 2$ (storage temperature) $+ \beta 3$ (mayonnaise pH$*$ storage temperature) $+ \beta 4$ (mayonnaise pH)$^2 + \beta 5$ (storage temperature)^2, where α is the intercept, and $\beta 1$–$\beta 5$ are estimated coefficients for each parameter.

The 'goodness-of-fit' of the equations was evaluated by the regression significance (p), correlation coefficient (R^2), bias factor $\left(B_f = 10^{\Sigma \log(\text{LPD or GR}_{predicted}/\text{LPD or GR}_{observed})/n}\right)$ and accuracy factor $\left(A_f = 10^{\Sigma |\log(\text{LPD or GR}_{predicted}/\text{LPD or GR}_{observed})|/n}\right)$. The B_f indicated that, on average, the predicted values were higher or lower than the observed values, while the A_f indicated the average closeness of the predicted values and the observed values (Ross, 1996). The mathematical equations were used to generate response surface plots to visually represent the effect of mayonnaise pH and storage temperature on the LPD and GR of *L. monocytogenes* in both salads.

3. Results and discussion

3.1. Growth of L. monocytogenes in egg salad and pasta salad

The pH of hard-boiled egg was 7.6. The pH of homogenized egg salad formulated with mayonnaise of pH 3.8, 4.2, 4.6, and 5.0 were pH 6.3, 6.7, 7.1, and 7.5, respectively. The initial levels of *L. monocytogenes* in egg salad were $\sim 10^3$ cfu/g. *L. monocytogenes* was able to grow in egg salad, regardless of the mayonnaise pH, during storage at 4, 8, and 12 °C (Figs. 1A–C). The cell counts of *L. monocytogenes* increased to $\sim 10^6$ cfu/g after 10 days at 4 °C (Fig. 1A), 5 days at 8 °C (Fig. 1B), and 3 days at 12 °C (Fig. 1C). The maximum cell counts of *L. monocytogenes* in egg salad were $\sim 10^8$ cfu/g at the end of the storage. Results showed that the growth of *L. monocytogenes* in egg salad was significantly promoted by higher storage temperatures, and the growth trend was not different in egg salads formulated with mayonnaise of different pH, indicating the mayonnaise pH may have no significant effect on the growth of *L. monocytogenes* in egg salad.

The pH of cooked pasta was 6.4. The pH of homogenized pasta salad formulated with mayonnaise pH of 3.8, 4.2, 4.6, and 5.0 were 4.7, 5.0, 5.2, and 5.7, respectively. *L. monocytogenes* was able to grow in all pasta salad during storage at 4, 8, and 12 °C (Fig. 1D–F). The initial levels of *L. monocytogenes* in pasta salad were approximately 10^3 cfu/g. The counts increased to approximately 10^6 cfu/g after storage for 21 days at 4 °C, 9 days at 8 °C, and 5 days at 12 °C. As observed in egg salad, *L. monocytogenes* grew more rapidly at higher storage temperatures. The slowest growth of *L. monocytogenes* was observed in pasta salad formulated with mayonnaise of pH 3.8 and 4.0 and stored at 4 °C. At 4 °C, the growth of *L. monocytogenes* increased as the mayonnaise pH increased (Fig. 1D). However, the effect of low mayonnaise pH on the growth of *L. monocytogenes* in pasta salad was not noticeable at storage temperatures of 8 and 12 °C (Figs. 1E–F). The maximum cell counts of *L. monocytogenes* were approximately 10^6 cfu/g in pasta salads formulated with mayonnaise pH 3.8 and 4.0 and stored at 4 °C, whereas the maximum cell counts were 10^{8-9} cfu/g for other mayonnaise pH and storage temperatures. Results showed that the storage temperature had an effect on the growth of *L. monocytogenes* in pasta salad, whereas the mayonnaise pH may only have effect on *L. monocytogenes* in salads stored at 4 °C.

Results for both salads showed that the storage temperature was the main factor that influenced the growth of *L. monocytogenes* in egg salad and pasta salad. The significant effect of storage temperature and lesser effect of mayonnaise pH, except in pasta salad stored at 4 °C, in influencing the growth of *L. monocytogenes* was also reported in studies examining seafood salad, ham salad and potato salad (Hwang and Tamplin 2005; Hwang, 2005). In this study, mayonnaise with lower pH (3.8 or 4.0) was able to slow the growth of *L. monocytogenes* in pasta salad at 4 °C, but this was not observed in egg salad. This implicates that the effect of mayonnaise pH is only evident when the combination of food component and storage temperature is less optimal for the growth of *L. monocytogenes*. Starch in cooked pasta may not provide sufficient nutrients for *L. monocytogenes* to grow. Therefore, the slow growth of *L. monocytogenes* observed in pasta salad was likely due to the limited nutrients, lower mayonnaise pH, and lower storage temperature (4 °C). When one of the growth condition improved, for example, at higher storage temperatures, the pH effect on the *L. monocytogenes* decreased. Comparison of the time for *L.*

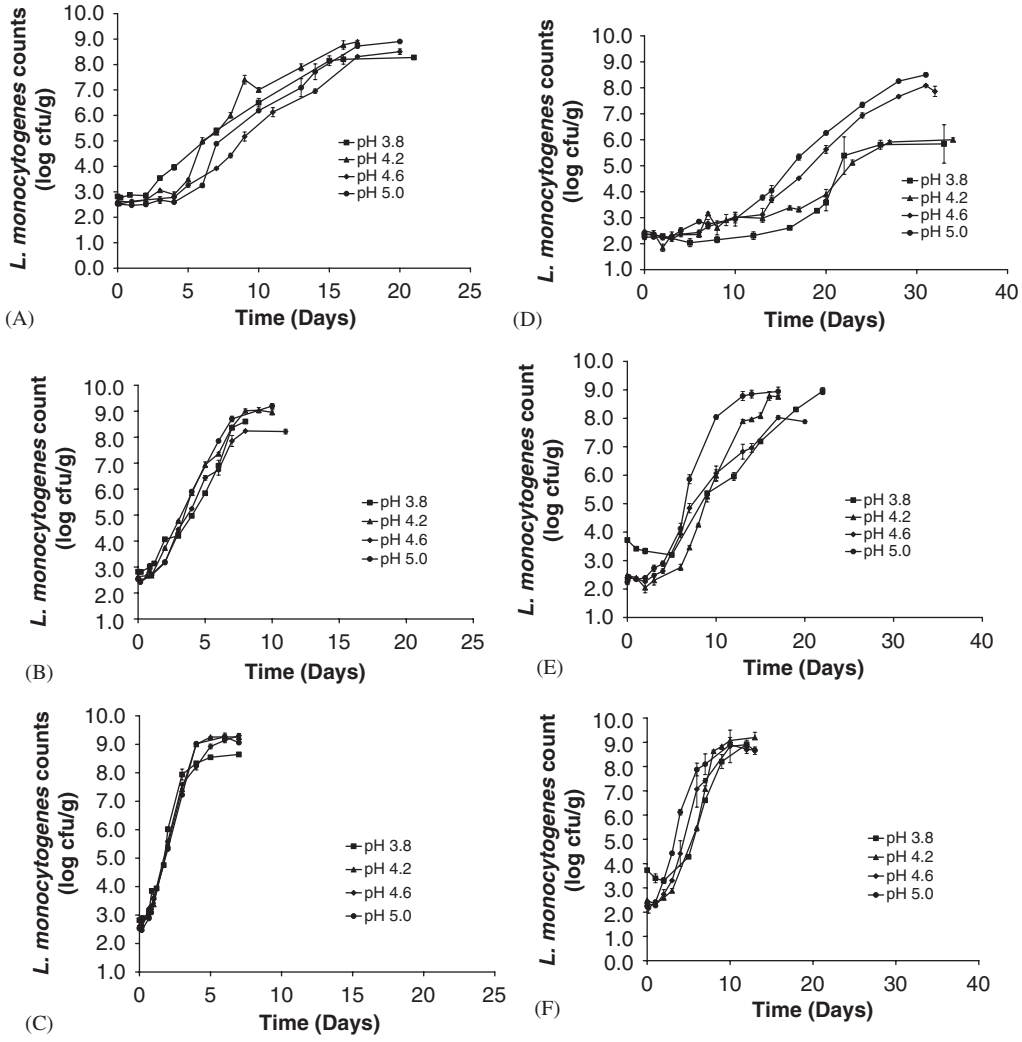

Fig. 1. The growth curves of *L. monocytogenes* in egg salad (A, 4 °C; B, 8 °C; C, 12 °C) and pasta salad (D, 4 °C; E, 8 °C; F, 12 °C) formulated with mayonnaise pH of 3.8 (■), 4.2 (▲), 4.6 (◆) and 5.0 (●). *L. monocytogenes* counts were the average of three replicates. Vertical bars indicate standard deviations.

monocytogenes to reach 10^6 cfu/g in both salads, the increase of cell counts of *L. monocytogenes* was more rapid in egg salad than in pasta salad, indicating that egg salad provided a better growth environment for *L. monocytogenes* than pasta salad. Nutritional data confirm that eggs contain 12.6% protein (15.9% in egg yolk), 10.6% total fat (26.5% in egg yolk) and 1.1% carbohydrates, whereas cooked pasta contains 5.15% protein, 1.1% fat and 24.93% carbohydrate (USDA, 2005a). Eggs, with higher protein contents and pH-buffering capacity, are likely to provide a more favorable growth environment for *L. monocytogenes* than the carbohydrate-rich pasta. An effect of the food components in salads on the behavior of *L. monocytogenes* was also observed in seafood salad, ham salad, and potato salad, in which *L. monocytogenes* was able to grow in seafood salad and ham salad, but not in potato salad (Hwang and Tamplin, 2005; Hwang, 2005). Erickson and Jenkins (1991) and Guentert et al. (2005) examined the behavior of *L. monocytogenes* in chicken salad. Erickson and Jenkins reported that *L. monocytogenes* was able to grow in home-made chicken salad stored at 4 °C, whereas Guentert et al. reported that *L. monocytogenes* was inactivated in chicken salad adjusted to pH 4.0–5.2 and stored at 5.0–21.1 °C. The latter attributed the different findings to the different salad formulation used in the studies, the interaction of pH, temperature, antimicrobial ingredients, the physical and chemical properties of the food, and the physical positioning of ingredients and micro-organisms throughout the food. Results from the present study support such observations. Regular mayonnaise (pH <4.0) and pH-adjusted mayonnaise (pH $\leqslant 5.0$) were shown to inactivate *L. monocytogenes* mainly due to the acidity in mayonnaise (Erickson and Jenkins, 1991; Glass and Doyle, 1991; Erickson et al., 1993; Hwang and Tamplin, 2005). Therefore, if cells of *L. monocytogenes* are introduced into salads after salads are made, the cells may present only in mayonnaise that coated the food components, and *L. monocytogenes* may not be able to grow. However, if the cells are introduced onto the surfaces of food components before the mayon-

naise is added, *L. monocytogenes* may be able to grow since the food components are likely to provide a better growth environment than mayonnaise. This study and our previous studies with seafood salad, ham salad and potato salad, in which *L. monocytogenes* cells were inoculated onto the food components before mixing with mayonnaise, indicates a significant effect of the physical and chemical properties of food components and the location of the micro-organisms, on surfaces of food components or in mayonnaise, in influencing the growth of *L. monocytogenes* in deli salads. This study indicates that *L. monocytogenes*, once it contaminates egg or pasta used for salad preparation, is able to grow in egg or pasta salads despite the addition of mayonnaise. Therefore, it is essential to ensure that egg or pasta used for salad preparation is handled properly to avoid *L. monocytogenes* contamination, and the finished products be kept at refrigerated temperature.

3.2. LPD and GR of *L. monocytogenes* in egg salad and pasta salad

The LPD (h) and GR ($\log_{10}$ cfu/h) of *L. monocytogenes* in egg salad are listed in Table 1. The LPD of *L. monocytogenes* in egg salad ranged from 34 to 85, 15 to 50, and 0 to 19 h at 4, 8, and 12 °C, respectively. The pH of mayonnaise did not produce a consistent change in the LPD. A longer LPD in salads formulated with mayonnaise of lower pH (3.8 or 4.2) was observed at 8 and 12 °C, but was not observed at 4 °C. The GR of *L. monocytogenes* in egg salad ranged from 0.0187 to 0.0318, 0.0387 to 0.0512, and 0.0694 to 0.1003 $\log_{10}$ cfu/h during storage at 4, 8, and 12 °C, respectively (Table 1). As expected, the GR were lower at lower storage temperatures. The effect of mayonnaise pH on the GR was not consistent in increasing or decreasing the GR of *L. monocytogenes* in egg salad.

The LPD of *L. monocytogenes* in pasta salad ranged from 210 to 430, 49 to 131, and 21 to 102 h, and the GR ranged from 0.0118 to 0.0350, 0.0153 to 0.0418, and 0.0453 to 0.0718 $\log_{10}$ cfu/h during storage at 4, 8, and 12 °C, respectively (Table 2). As observed in egg salad, the LPD of *L. monocytogenes* in pasta salad were generally longer and the GR were lower at lower storage temperatures.The effect of lower mayonnaise pH in increasing LPD was observed in pasta salad stored at 4 and 12 °C (Table 2). The effect of mayonnaise pH was not consistent in increasing or decreasing the GR of *L. monocytogenes* in both salads (Table 2).

Results showed that the LPD and GR of *L. monocytogenes* in both salads were mainly affected by the storage temperature. *L. monocytogenes* had longer LPD and lower GR in pasta salad than in egg salad, indicating that pasta provided a less favorable growth environment than egg for *L. monocytogenes*.

Table 1
Means (standard deviations) of lag-phase durations (LPD, h) and growth rates (GR, log cfu/h) of *L. monocytogenes* in egg salad stored at 4, 8, and 12 °C

Temperature (°C)	Mayonnaise pH	LPD (h)[a]		GR (log cfu/h)[a]	
4	3.8	33.8 (5.1)	C	0.0191 (0.0004)	G
	4.2	80.8 (2.0)	A	0.0318 (0.0007)	F
	4.6	84.5 (1.3)	A	0.0187 (0.0003)	G
	5.0	77.4 (7.2)	A	0.0217 (0.0011)	G
8	3.8	49.5 (5.2)	B	0.0439 (0.0026)	E
	4.2	14.9 (1.9)	D	0.0415 (0.0004)	E
	4.6	23.1 (2.9)	D	0.0387 (0.0018)	E
	5.0	34.2 (1.7)	C	0.0512 (0.0003)	D
12	3.8	19.2 (2.4)	D	0.1003 (0.0067)	A
	4.2	17.0 (0.4)	D	0.0889 (0.0008)	B
	4.6	0.0 (0.0)	E	0.0694 (0.0001)	C
	5.0	15.3 (1.2)	D	0.0869 (0.0021)	B

[a]Means of triplicate samples. Means in the column with different letters are significantly different ($P<0.05$).

Table 2
Means (standard deviations) of lag phase durations (LPD, h) and growth rates (GR, log cfu/h) of *L. monocytogenes* in pasta salad stored at 4, 8, and 12 °C

Temperature (°C)	Mayonnaise pH	LPD (h)[a]		GR (log cfu/h)[a]	
4	3.8	429.8 (33.2)	A	0.0350 (0.0185)	BCD
	4.2	345.0 (11.2)	B	0.0118 (0.0007)	E
	4.6	263.4 (19.7)	C	0.0149 (0.0007)	E
	5.0	209.7 (25.8)	D	0.0143 (0.0010)	E
8	3.8	110.4 (1.6)	E	0.0153 (0.0001)	E
	4.2	131.0 (4.8)	E	0.0328 (0.0024)	CD
	4.6	49.1 (12.3)	F	0.0190 (0.0020)	DE
	5.0	94.2 (1.2)	E	0.0418 (0.0004)	BC
12	3.8	02.1 (4.2)	E	0.0453 (0.0041)	BC
	4.2	03.8 (2.1)	E	0.0718 (0.0017)	A
	4.6	52.3 (9.3)	F	0.0487 (0.0008)	B
	5.0	20.7 (6.7)	F	0.0492 (0.0091)	B

[a]Means of triplicate samples. Means in the column followed by the different letters are significantly different ($P<0.05$).

3.3. Fitting of LPD and GR equations and goodness-of-fit

The mathematical equations that describing the LPD and GR of *L. monocytogenes* in egg salad and pasta salad as a function of mayonnaise pH, storage temperature, and their interaction are:

Egg salad:

$$\begin{aligned}\text{LPD (h)} = {} & 15.5 + 4.6(\text{mayonnaise pH}) + 4.78(\text{mayonnaise pH})^2 \\ & + 4.9(\text{storage temperature}) + 0.7(\text{storage temperature})^2 \\ & - 5.1(\text{mayonnaise pH}*\text{storage temperature}). \\ & (P<0.001,\ R^2 = 0.79)\end{aligned}$$

$$\begin{aligned}\text{GR}(\log_{10}\text{cfu/h}) = {} & 0.3228 - 0.147(\text{mayonnaise pH}) \\ & + 0.0179(\text{mayonnaise pH})^2 \\ & + 0.0046(\text{storage temperature}) \\ & + 0.0007(\text{storage temperature})^2 \\ & - 0.0017(\text{mayonnaise pH}*\text{storage temperature}). \\ & (P<0.001,\ R^2 = 0.94).\end{aligned}$$

Pasta salad:

$$\text{LPD (h)} = 1697.6 - 251.4(\text{mayonnaise pH}) + 6.0(\text{mayonnaise pH})^2 - 180.4(\text{storage temperature}) + 5.8(\text{storage temperature})^2 + 13.0(\text{mayonnaise pH} * \text{storage temperature}).$$

$(P < 0.001,\ R^2 = 0.94)$

$$\text{GR } (\log_{10} \text{cfu/h}) = 0.0571 - 0.0091(\text{mayonnaise pH}) + 0.0005(\text{mayonnaise pH})^2 - 0.0070(\text{storage temperature}) + 0.0006(\text{storage temperature})^2 + 0.0006(\text{mayonnaise pH} * \text{storage temperature}).$$

$(P < 0.001,\ R^2 = 0.69)$.

Table 3
Parameter estimates (coefficients) and their significance levels for mayonnaise pH, storage temperature (temp), and their interaction

Parameter	Egg salad				Pasta salad			
	LPD		GR		LPD		GR	
	Estimate	*P*	Estimate	*P*	Estimate	*P*	Estimate	*P*
Intercept	15.5	0.9569	0.3228	0.0370	1697.6	0.0208	0.0571	0.8212
pH	4.6	0.9713	−0.147	0.0347	−251.4	0.4233	−0.0091	0.9355
pH∗pH	4.78	0.7445	0.0179	0.0243	6.0	0.8641	0.0005	0.9701
Temp	4.9	0.5746	0.0046	0.3068	−180.4	<0.0001	−0.0070	0.3732
Temp∗temp	0.7	0.0396	0.0007	0.0002	5.8	<0.0001	0.0006	0.0400
pH∗temp	−5.1	0.0031	−0.0017	0.0486	13.0	0.0032	0.0006	0.6859

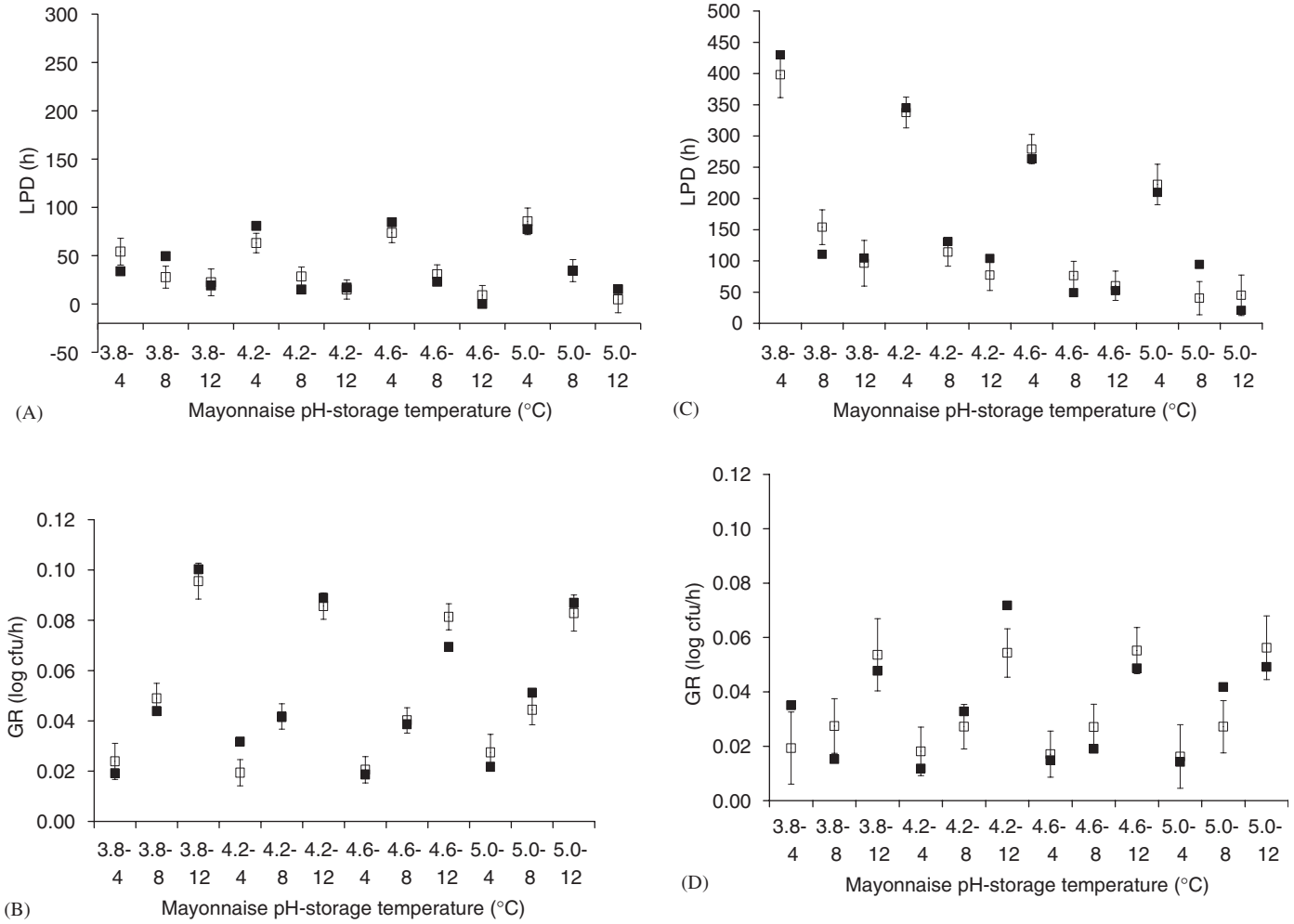

Fig. 2. The estimated values (□) with corresponding 95% confidence limits (vertical bars), and the observed values (■) of LPD and GR of *L. monocytogenes* in egg salad (A, LPD; B, GR) and pasta salad (C, LPD; D, GR).

The equations were significant ($P<0.001$) and sufficient ($R^2 = 0.69 - 0.94$) in estimating the values of LPD and GR of *L. monocytogenes* obtained from the experiments. The levels of significance of mayonnaise pH, storage temperature, and their interaction in influencing the LPD and GR of *L. monocytogenes* in egg salad and pasta salad are shown in Table 3. The LPD of *L. monocytogenes* in egg salad was significantly affected by the storage temperature and the interaction of storage temperature and mayonnaise pH, whereas the GR was significantly affected by mayonnaise pH, storage temperature, and their interaction. The LPD of *L. monocytogenes* in pasta salad was significantly affected by the storage temperature and the interaction of storage temperature and mayonnaise pH, whereas the GR was only significantly affected by the storage temperature. The estimated LPD from the equations of egg salad and pasta salad would be, on average, 67% ($A_f = 1.67$) and 31% ($A_f = 1.31$), respectively, higher ($B_f > 1.00$) than the observed values. The estimated GR would be 15% ($A_f = 1.15$) and 34% ($A_f = 1.34$) higher ($B_f > 1.00$) than the observed values in egg salad and pasta salad, respectively. The estimated LPD, GR and their 95% confidence limits obtained from the equations, and the observed values for each of the mayonnaise pH and storage temperature are shown in Fig. 2. It is reasonable to assume that the observed value is not significantly different from the estimated value when the observed value is within the 95% confidence limits of the estimated value. In egg salad, 8 out of the 12 observed LPD values (67%) and 10 out of the 12 observed GR values (83%) are within the 95% confidence limits of the estimated values (Figs. 2A and B), whereas in pasta salad there are 11 out of 12 (92%) for LPD and 8 out of 12 (67%) for GR (Figs. 2C and D). The LPD equation for egg salad is more satisfactory in estimating the LPD of *L. monocytogenes* in egg salad formulated with mayonnaise pH of 4.6 and 5.0 (Fig. 2A). In general, the GR equation of egg salad is sufficient to estimate the observed values in all salads except those formulated with mayonnaise of pH 4.2 and stored at 4 °C, and mayonnaise of pH 4.6 and stored at 12 °C (Fig. 2B). In pasta salad, the equation is sufficient to estimate the LPD in all salads except for those formulated with mayonnaise of pH 3.8 and stored at 8 °C, and pH of 5.0 and stored at 8 °C (Fig. 2C). The GR equation for pasta salad is not satisfactory in estimating the observed GR in pasta salads formulated with mayonnaise of pH 3.8 and stored at 4 or 8 °C, pH 4.2 at 12 °C, and pH 5.0 at 8 °C (Fig. 2D). These information indicate the conditions for both salads that the equations are able to reasonably predict the LPD and GR, therefore, the equations may be used selectively to estimate the growth of *L. monocytogenes* in egg salad and pasta salad stored at refrigerated and abuse temperatures.

Response surface plots derived from the equations are shown in Fig. 3. The plots confirm that the storage temperature is the main factor that influences the LPD and GR of *L. monocytogenes* in both salads, in which *L. monocytogenes* has longer LPD and lower GR at lower storage temperatures. Theoretically, *L. monocytogenes* is

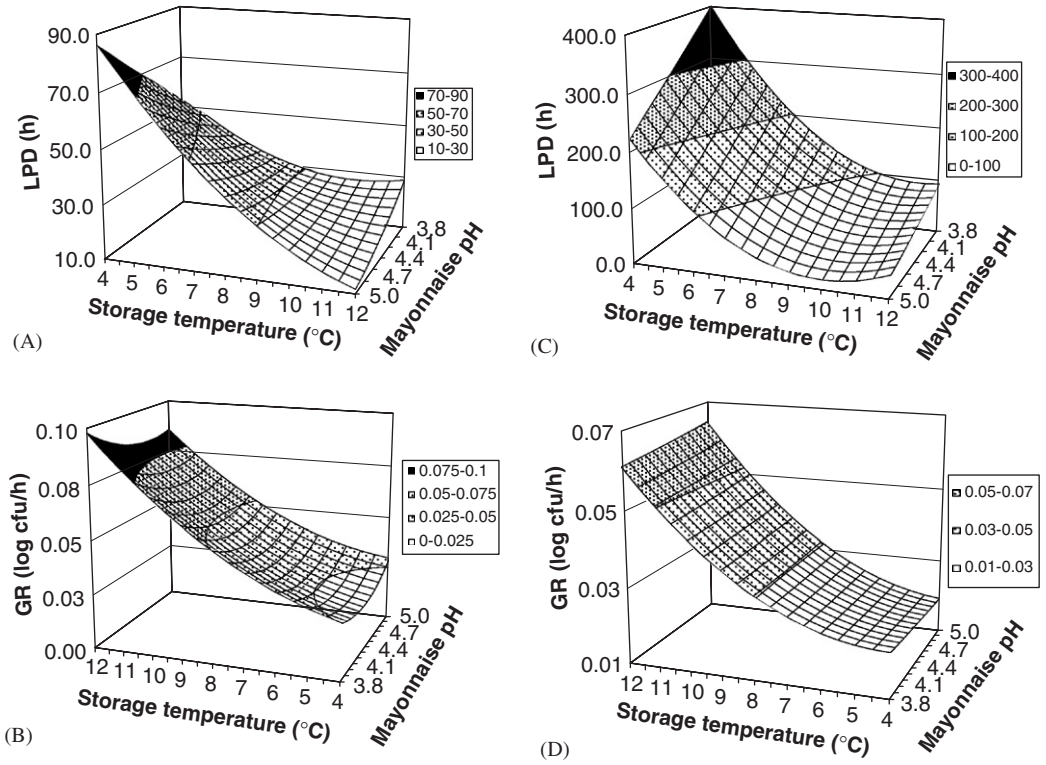

Fig. 3. Response surface plots of the mathematical equations for the LPD and GR of *L. monocytogenes* in egg salad (A, LPD; B, GR) and pasta salad (C, LPD; D, GR).

expected to have longer LPD and lower GR in environments with lower pH. In this study, the effect of mayonnaise pH on LPD or GR of *L. monocytogenes* is not consistent. In egg salad, longer LPD are shown only at lower mayonnaise pH and at storage temperature above 8 °C, while lower GR are shown in salads with mayonnaise pH of 4.2 and 4.6 (Figs. 3A and B). In pasta salad, longer LPD are shown at lower pH at storage temperatures below 9 °C, while the GR are similar among the various mayonnaise pH tested (Figs. 3C and D). The inconsistent effect of mayonnaise pH on the LPD and GR of *L. monocytogenes* indicates that a more complex environment exists at the junction of the food components and mayonnaise. For example, the pH-buffering capacity of food components and moisture equilibration between the food components and mayonnaise are likely to influence the pH and water activity at the interface of the food components and mayonnaise. The effect of a changing environment at the junction of two environments on the behavior of micro-organisms needs further investigation.

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